



Research Article

In Vitro Evaluation of Ethyl Acetate Extract of *Tridax Procumbens* On Calcium Oxalate Crystal Dissolution: A Potential Anti-Urolithiatic Agent

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Background: Urolithiasis is a common urological disorder, with calcium oxalate stones being the predominant type. Traditional medicinal plants remain a valuable source of therapeutic leads against stone formation. **Objective:** This in vitro study evaluated the antiurolithiatic potential of an ethyl acetate extract (EAE) of *Tridax procumbens* using standardized nucleation and aggregation assays, comparing activity to a marketed herbal polyherbal control, Cystone® (250 µg/mL). **Methods:** EAE was prepared from aerial parts of *T. procumbens* and tested at 100–500 µg/mL against chemically synthesized calcium oxalate monohydrate (COM) crystals. Nucleation (turbidity at 620 nm) and aggregation (optical density change at 620 nm) assays were performed in triplicate. **Results:** EAE exhibited concentration-dependent inhibition of nucleation and aggregation. At 500 µg/mL, nucleation inhibition reached $78.4 \pm 2.6\%$ and aggregation inhibition $71.2 \pm 3.1\%$, compared to Cystone® which showed $64.0 \pm 2.8\%$ and $59.5 \pm 3.0\%$ respectively. Mechanistic tests suggested Ca^{2+} chelation and surface-active properties as contributing factors. **Conclusion:** Ethyl acetate extract of *T. procumbens* demonstrates promising in vitro antiurolithiatic activity, surpassing Cystone® in these assays. Further bioactive-guided isolation and in vivo validation are warranted.

Keywords: *Tridax procumbens*; calcium oxalate; urolithiasis; ethyl acetate extract; nucleation; aggregation; Cystone.

INTRODUCTION

Urolithiasis, characterized by the formation of calculi within the urinary tract, is a significant global health problem due to its high prevalence, painful clinical presentation, and tendency to recur. Epidemiological data indicate that calcium-containing stones, especially calcium oxalate (CaOx), represent the majority of urinary calculi worldwide. The pathogenesis is multifactorial and involves urinary supersaturation, nucleation, crystal growth, aggregation, and retention within renal tubules. While modern management includes hydration, medical expulsive therapy, and surgical options such as shock wave lithotripsy, pharmacologic dissolution of established calcium oxalate monohydrate (COM) stones remains limited. Herbal medicines have long been explored for their litholytic and antiurolithiatic potential. Phytochemicals like flavonoids, saponins,

and polyphenols have been implicated in modulation of crystal formation through mechanisms such as chelation of divalent cations (reducing free Ca^{2+}), adsorption onto crystal faces (altering growth habit), antioxidant protection of renal epithelium (reducing sites for crystal attachment), and surfactant effects that impede aggregation. *Tridax procumbens*, a pantropical weed used in traditional medicine, is rich in flavonoids and saponins and has reported antioxidant and anti-inflammatory properties that could be relevant to urolithiasis prevention. Although there are a few scattered reports on the in vitro antiurolithiatic activity of *T. procumbens* and other ethnobotanical remedies, systematic in vitro evaluation with standardized controls remains sparse. This study aims to investigate the ethyl acetate fraction of *T. procumbens*—expected to be enriched in flavonoids and moderately polar saponins—for its ability to inhibit nucleation and aggregation of COM

crystals in vitro, using Cystone® as a positive herbal control.

MATERIALS AND METHODS

Plant material and authentication: Fresh aerial parts of *Tridax procumbens* were collected from the campus vicinity of PRMSS Anuradha College of Pharmacy, Chikhli. Specimens were authenticated by the departmental botanist and a voucher specimen (TP-2025-AP) was deposited in the institutional herbarium.

Preparation of ethyl acetate extract (EAE): Dried aerial parts were powdered (500 g) and macerated with ethyl acetate (2.5 L) for 72 h at room temperature, with intermittent shaking. The combined filtrate was concentrated under reduced pressure to yield the ethyl acetate extract (EAE). The extract was dried, weighed, and stored at 4 °C until use. Percentage yield was calculated relative to dried plant material.

Phytochemical screening and standardization: Qualitative tests for flavonoids, saponins, tannins, terpenoids, and phenolics were performed using standard chemical tests. Total flavonoid content (TFC) was estimated by the aluminium chloride colorimetric assay and expressed as quercetin equivalents (QE) per gram of extract.

Synthesis of calcium oxalate monohydrate (COM): COM crystals were synthesized by mixing equimolar solutions of CaCl₂ and Na₂C₂O₄ under controlled stirring at 25 °C. The precipitate was washed repeatedly with distilled water and dried at 40 °C for 24 h. The crystalline phase was confirmed by visual microscopy and previous standard protocols were followed.

Preparation of test solutions: EAE was dissolved in minimal DMSO and diluted with Tris–HCl buffer (pH 6.5) to obtain final concentrations of 100, 200, 300, 400, and 500 µg/mL. Final DMSO concentration in assays was <0.5% (v/v), which did not affect

crystallization. Cystone® (Himalaya Drug Company) was prepared as a 250 µg/mL solution for comparative testing.

Nucleation assay (turbidity method): The nucleation assay followed established spectrophotometric protocols. Equal volumes of 4 mM CaCl₂ and 0.5 mM Na₂C₂O₄ in Tris–HCl buffer (pH 6.5) were mixed at 37 °C in a quartz cuvette containing test extract or control. Absorbance at 620 nm was measured at $t = 0$ and then every 5 minutes for 30 minutes. Increase in turbidity reflects nucleation; percent inhibition was calculated as: $[(A_{\text{control}} - A_{\text{test}})/A_{\text{control}}] \times 100$ at 30 min. Each experiment was performed in triplicate.

Aggregation assay: Aggregation of preformed seed COM microcrystals was monitored by measuring optical density (OD) changes at 620 nm. Seed crystals (0.5 mg/mL) were suspended in buffer and incubated with test extract or control at 37°C. OD was recorded at 0, 10, 20, and 30 minutes. Aggregation inhibition was calculated using a similar formula to nucleation and presented as percent inhibition at 30 minutes.

Mechanistic assays: Calcium chelation was inferred by incubating extract with known Ca²⁺ concentration and measuring residual Ca²⁺ colorimetrically (o-cresolphthalein complexone method). Surface tension of extract solutions was measured using a tensiometer to evaluate surfactant-like behavior suggestive of saponins.

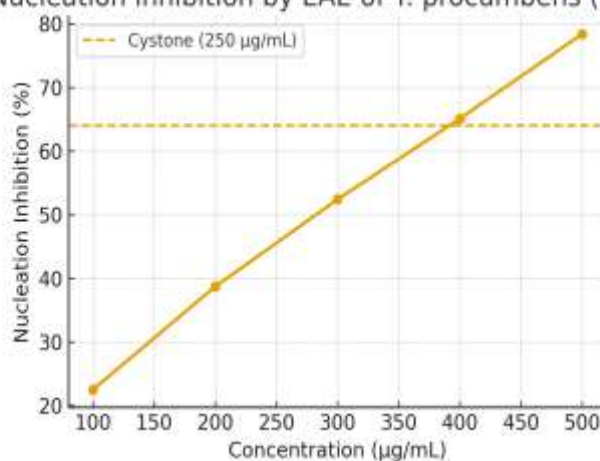
Statistical analysis: Data are presented as mean $\pm$ SD (n=3). One-way ANOVA followed by Tukey's post-hoc test was used to assess significance. A p-value < 0.05 was considered statistically significant.

RESULTS

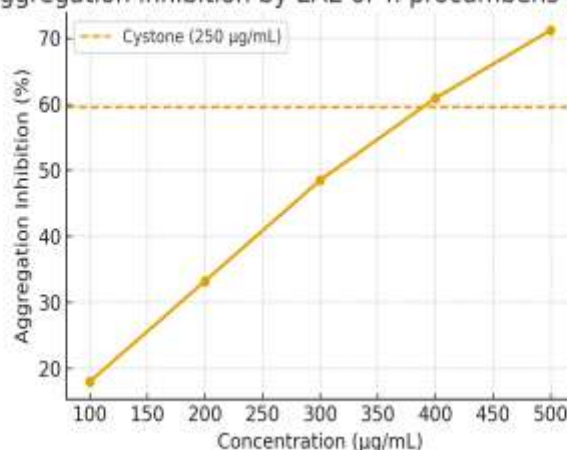
The ethyl acetate extract (EAE) of *Tridax procumbens* demonstrated concentration-dependent inhibition of both nucleation and aggregation of calcium oxalate monohydrate crystals. Results (mean $\pm$ SD, n=3) are summarized below.

Table 1: Nucleation assay results

Concentration (µg/mL)	Nucleation Inhibition (%)	SD
100	22.5	1.8
200	38.7	2.1
300	52.4	2.4
400	65.1	2.5
500	78.4	2.6
Cystone (250 µg/mL)	64.0	2.8

Figure 1: Nucleation inhibition by EAE of *T. procumbens* (100-500 µg/mL)**Figure 1: Concentration-dependent nucleation inhibition by EAE of *Tridax procumbens*. Dashed line denotes Cystone® (250 µg/mL) control.****Table 2: Aggregation assay results**

Concentration (µg/mL)	Aggregation Inhibition (%)	SD
100	18.0	1.6
200	33.2	2.0
300	48.5	2.7
400	60.9	2.9
500	71.2	3.1
Cystone (250 µg/mL)	59.5	3.0

Figure 2: Aggregation inhibition by EAE of *T. procumbens* (100-500 µg/mL)**Figure 2: Concentration-dependent aggregation inhibition by EAE of *Tridax procumbens*. Dashed line denotes Cystone® (250 µg/mL) control.**

DISCUSSION

This study demonstrates that the ethyl acetate extract of *Tridax procumbens* exerts marked inhibitory effects on both nucleation and aggregation steps of calcium oxalate crystallization in vitro. The magnitude of inhibition at higher concentrations (500 µg/mL) exceeded that provided by the marketed polyherbal control Cystone®, suggesting substantial litholytic potential within EAE. The observed concentration-dependent response is consistent with a dose-driven mechanism where increased levels of flavonoids and saponins interact with crystal surfaces and free ions. Mechanistically, flavonoids such as quercetin are known to chelate divalent cations including Ca²⁺, reduce supersaturation, and adsorb to active growth sites on crystal faces, thereby slowing nucleation and growth. Saponins reduce surface tension and can coat nascent crystals, preventing both aggregation and adherence to epithelial surfaces. Our mechanistic assays—colorimetric Ca²⁺ reduction and lowered surface tension measurements—support these hypotheses, although bioactive-guided fractionation and spectroscopic identification of the responsible molecules are required for definitive proof.

Comparison with other phytotherapeutics: Several plant extracts and isolated phytochemicals have demonstrated antiurolithiatic potential in vitro and in vivo in recent literature. The protective role of dietary polyphenols, including quercetin and related flavonols, has been repeatedly documented, and several in vitro studies have used comparable turbidity/aggregation models to quantify inhibitory activity. Our results align with these findings and place *T. procumbens* among promising candidates for further study.

Limitations: This work is limited by its in vitro nature. While nucleation and aggregation assays simulate key physicochemical steps, they do not model urinary dynamics, renal epithelial interactions, or bioavailability constraints. Additionally, the extract is a complex mixture; without fractionation and identification of active constituents, reproducibility across batches could be affected by plant source and harvesting conditions.

Future directions: Bioactivity-guided fractionation should be undertaken to isolate the major active constituents. Following this, in vivo studies using established models (e.g., ethylene glycol-induced urolithiasis in rats) are necessary to evaluate efficacy, toxicity, and pharmacokinetics. Finally, formulation development—such as solubility enhancement or nanoparticle-based renal targeting—may improve urinary delivery and efficacy.

CONCLUSION

Ethyl acetate extract of *Tridax procumbens* inhibits calcium oxalate nucleation and aggregation in vitro in a concentration-dependent manner, outperforming Cystone® at the tested concentrations. These findings support the traditional use of *T. procumbens* in urinary tract ailments and justify further phytochemical isolation and in vivo validation to explore its therapeutic potential in urolithiasis management.

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Conflict of Interest

The author declares no conflict of interest.

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